SUN YAT-SEN UNIVERSITY,

ZHONGSHAN SCHOOL OF MEDICINES

APPLICATION FORM FOR ETHICAL REVIEW OF WELFARE AND ETHICS OF EXPERIMENTAL ANIMALS

<table>
<tr><td colspan="4">Protocol name: The therapeutic effect of the over-expression EGF on E.coli in IBD mice model</td></tr>
<tr><td colspan="4">Protocol source: General Program, The National Natural Science Foundation of China</td></tr>
<tr><td colspan="2">Principle investigator:
Xiang Peng</td><td colspan="2">Name of unit: Key Laboratory for Stem Cells and Tissue Engineering</td></tr>
<tr><td colspan="2">Title Professor</td><td>Tel. +86 13543445898</td><td>E-mail: xiangp@mail.sysu.edu.cn</td></tr>
<tr><td colspan="4">Experimental animal using permission No.: SYXK（粤）2015-0107</td></tr>
<tr><td colspan="4">Experiment schedule:: July 1st, 2016 至 October 1st, 2016</td></tr>
<tr><td colspan="4">Please provide the name and the permission number of previous censored projects and elaborate which are similar or identical to this project.
None</td></tr>
<tr><td colspan="4">Is the project correlate to previous censored projects? If so, write down the project name and the approval number.
No</td></tr>
<tr><td rowspan="2">Experimental animal</td><td>Source:
Sun Yat-sen experimental animal center</td><td colspan="2">Breed strain: BALB/c mice</td></tr>
<tr><td>Level: SPF (√)
Normal ( )</td><td colspan="2">Number of BALB/c mice: 10
(≈ 10)</td></tr>
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The purpose, method, significance and feasibility of the experiments.

Purpose:

The project is aimed to confirm the therapeutic effect of the over-expression EGF on E.coli in IBD mice model. After engineered modified, E.coli products the EGF protein, which will be purified and inserted into the colon.

Method:

1. Build a plasmid containing gene we desired. A vector, pET28a is used to build the plasmid. Insert gene segment of EGF to the cloning region of pET28a. Add 6*his tag to the terminal of the segment. We use *Escherichia coli* BL21(DE3) as the expression strain, which is widely used in expression of gene that is promoted by T7 promoter. We use *Escherichia coli* BL21(DE3) as the expression strain, which is widely used in expression of gene that is promoted by T7 promoter. Using IPTG to induct our bacterial suspensions when logarithmic growth of microbes is nearly finished; Centrifuge the bacterial suspensions. Suspend the precipitate by PBS solution. Purify the protein we desired by Ni-charged MagBeads.

2. We select the most suitable temperature (24℃) for the mice. The light is operated as the circadian rhythms and the noise and vibration will be well controlled. Day 1, carefully shave a 1cm×1cm filed on the back between the shoulders of the mouse using an electric razor. While holding the mouse with one hand, smear the shaved abdominal skin with presensitization solution (150ul/20g), which is the mixture of acetone, olive oil and TNBS in 16:4:5 scale. Control mice are treated with presensitization solution without TNBS. Leave the mice until day 7. On day 7, absolute diet is applied to the mice apart from the water. One day 8, anesthetize the mouse by intraperitoneal injection of 4% chloral hydrate (150ul/20g). Insert the catheter into the colon 4cm proximal to the anus and slowly administer 150ul/20g TNBS/alcohol solution into the colon. Keep the mice with hand down for 60s before returning to the cage. Concerning the damaged colon, we take the jelly for the mice to lessen the pain after modelling. The number of the mice per cage decrease from 5 to 2 after modelling and the packing will be altered every 3 days.

3. Weight the mouse after day 7 every morning and assess the mice as the standard below:

Score	Weight lost (%)	Stool form	Hematochezia
0	0	Normal	Normal
1	1-	Loose(+)	Fecal occult blood(+)
2	5-	Loose(+ +)	Fecal occult blood(+ +)
3	10-	Liquidness(+)	Hematochezia (+)
4	>15	Liquidness(+ +)	Hematochezia(+ +)

4. Dissolve 5 μg of recombinational EGF in 25 ml saline. Insert 150ug/20g EGF solution into the colon. The colon length, DAI score and the concentration of the cytokines will be performed using SPSS.

5. All the animals will be sacrificed by cervical dislocation. Once the mice are in great pain, they will be sacrificed to reduce the suffering. The process of feeding, operation and management are in conformity with the detail rules for the implementation of laboratory biological safety management and experimental animal welfare ethics principle.

Significance:

Epidermal growth factor (EGF) is a grow factor that stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR. The biological effects of salivary EGF include healing of oral and gastroesophageal ulcers, inhibition of gastric acid secretion, stimulation of DNA synthesis as well as mucosal protection from intraluminal injurious factors such as gastric acid, bile acids, pepsin, and trypsin and to physical, chemical and bacterial agents. In 2003, Dr.Sinha A treated the IBD patients with EGF and this clinic trail had great therapy effect. In animal experiments, We hope to confirm that the EGF molecular secreted by synthetic biology engineered E. coli can achieve the same effect, thus a universal IBD treatment can be provided.

Feasibility:

1. Full foundation.

Our project is based on sufficient article reading and clinical information about the evidence the biological function of EGF, which can bind with EGFR and active the downstream MAPK 与 PI-3K pathways to affect the DNA synthesis, transcription and cell proliferation. Furthermore, EGF can decrease the bacterial translocation, protecting intestinal mechanical barrier and chemical barrier. Accordingly, the damaged colon can be repaired.

2. Lab.

The lab condition and staff guarantee the actualization of the project. Our research is supported by Key Laboratory for Stem Cells and Tissue Engineering which is a center for Stem Cell Biology and Tissue Engineering, supported by Zhongshan School of Medicine, Sun Yat-sen University, Ministry of Education. With a Safety Level 2 laboratory of approximate 3500m^2 and equipment worth over 15 million, we are equipped with important and supportive platforms, such as stem cells bank and stem cell preclinical primate evaluation platform, as well as necessary equipment for the project such as BD-Influx flow cytometry、 Illumina Solexa GAIIX gene sequencing instrument and laser scanning confocal microscope.

Feeding situation of experimental animals	Fed and managed by Sun Yat-sen experimental animal center
The design of the animal experiment	Experimental grouping: IBD MODEL（♂ 10）: IBD model with 10 BALB/c mice will be established and divided into 2 experiments: (1) 5 mice will be treated with EGF clyster in this experiment. (2) 5 mice will be treated with saline clyster as control group.
	Administration mode: intragastric administration (√) subcutaneous injection ( ) intraperitoneal injection ( ) intravenous injection (√) others ( ) Note if 'other' is chosen:
	Executing manner: cervical dislocation (√) overdose of anesthetic ( ) others ( ) Note if 'other' is chosen:
	The management of dead bodies: sanity treatment (√) others( ) Note if 'other' is chosen:
The embodiment of "3R" principle	Replacement: In our design, we choose to culture bone marrow derived mesenchymal stem cell in vitro to replace living animals.

in designing the experiments	Reduction: In our design, we have reduced the number of animals used. We will establish IBD model of BALB/c mice which will be divided into two experiments. The first group is treated with EGF clyster and the second group is treated with saline. The colon length, DAI score and the concentration of the cytokines will be performed using SPSS.
	Refinement: All the experiment technique required in our project, such as anesthesia, surgical operations and function test has been fully mastered. At the same time, the experimental technical route has been delicately designed and selected in order to reduce discomfort and suffer of experimental animals which will promise the repeatability of animal experiment.

Announcement: I will consciously abide by the experimental animal welfare ethics principle and agree to accept the supervision as well as inspection of commission and inspector of laboratory manager. If I violate the commitment or stipulation, I will voluntarily accept punishment.

Signed (or stamped) by the announcer: Zhou Longyuan Date: 1st, August, 2016

Review opinion of the declaring unit:

Agree

Signed (or stamped) by the head executive investigator: D.P.Xing

Date 10th, August, 2016

Instruction:

1. The principle and the head executive investigators of the project and the leader of cooperative departments are required to sign in the announcer's signature column.
2. materials for review shall be submitted with the application (a copy of electronic as well as written document). The issues involving animal welfare and ethics, such as significance, necessities, purpose and feeding management of experimental animal, the experiment operation, observation steps and methods need expatiation. Additional pages are acceptable.
3. Please print double-sided.